World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease

Part 2

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This is the second of four reports on the 8th Annual World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease (WCIRDC), 4–6 November 2010, Los Angeles, California.

GENES AND INSULIN RESISTANCE—Robert Lane (Salt Lake City, UT) discussed the association of early life stresses with heart disease, cancer, Alzheimer’s disease, renal disease, and diabetes, leading causes of morbidity and mortality in the U.S. The most common stressor, intratruncine growth retardation (IUGR), is strongly associated with insulin resistance (IR) and with cardiovascular disease (CVD). The classic example of this phenomenon comes from study of the Dutch famine at the end of World War II, which occurred during a 5-month period when the Nazi occupiers retaliated for a railroad strike. Caloric intake dropped from 1,800 to 600 per day and after liberation increased to >2,000. The Dutch Famine Birth Cohort Study found that in utero exposure during the first trimester led to CVD, hypertension, dyslipidemia, and obesity; exposure during the second trimester to pulmonary disease; and exposure during the third to diabetes, depression, schizophrenia, and antisocial personality disorder, leading Lane to conclude that “there isn’t a very simple relationship between early life stressors and disease.” Subsequent studies have examined the effect of ponderal index (weight-height relationship at birth) on adult insulin sensitivity. In Uppsala, Sweden, men in the lowest quintile of the ponderal index had increased likelihood of IR at age 60 years. Lane pointed out that that in the U.S., 12.5% of infants are now born prematurely so that adult complications of prematurity “are going to start hitting your practices.” Prematurity is associated with neurodevelopmental delay, attention deficit disorder, sudden infant death syndrome, chronic lung disease, CVD, and many other conditions. The likelihood of hypertension in young adulthood is strongly related to prematurity, adjusted for current BMI, maternal age, parity, parental education, and occupation.

A mechanism for the effect of IUGR involves cell number effects, with abnormal differentiation of neural stem cells, proliferation of lung mesenchymal cells, and increased nephron apoptosis during formative stages. Another potential explanation is found in concepts of epigenetics, a notion of maternal imprinting leading to developmentally regulated gene expression, with implications for adaptation to the environment. DNA should, Lane noted, be considered three dimensional rather than linear. Lane pointed out that DNA is compacted through its association with histone proteins to form nucleosomes embedded in chromatin, with cofactors increasing or decreasing transcription, involving histone coding. There are eight histones for every 200–250 DNA base pairs, each having a “tail” that can be acetylated or methylated, changing rates of gene transcription during life, with all these modifications potentially “storing [epigenetic] information in your chromatin structure.”

Insulin-like growth factor (IGF)-1 control may be seen as a paradigm of epigenetic perinatal adaptation, with levels affected by IUGR and other early life events. IGF-1 is involved in a number of postnatal processes affected by early life events, including IR, obesity, and chronic lung disease. The IGF-1 gene is relatively simple, with 6 exons. Alternative RNA transcripts from exon 1 or exon 2 with or without transcripts of exon 5 lead to several related IGF-1 proteins. IUGR affects the IGF-1 histone 3 code along the length of the gene (1), appears to involve modulation of levels rather than simple on/off effects, and shows a degree of sex variability.

Several early life epigenetic biomarkers have been used to predict later life disease. In another study of Dutch famine offspring at age 60 years, levels of interleukin-10, ATP binding cassette transporter 1, guanine nucleotide binding protein, and other markers were elevated and levels of proteins formed by insulin induced genes were reduced. DNA methylation levels were higher in offspring of supplemented than in offspring of unsupplemented mothers. Another study of IUGR showed hepatic nuclear factor (HNF)-4α effects. In a study of suicide victims with and without childhood abuse, the former had increased localized neuronal DNA methylation. Almost all dietary components can change DNA methylation, including calorie and macro-nutrient content. Learning how epigenetic changes are used to integrate early life stressors will shed light on the mechanism by which genes generate a continuum of responses, but it will require great caution to develop epigenetic treatment approaches, recognizing that increasing the expression of a “good” gene may have unrecognized effect on “bad” genes. At present, Lane continued, “our technology limits us to just looking at very simple things, but there’s a wealth of information . . . if we just learn how to mine it.”

Jerome Rotter (Los Angeles, CA) discussed the genetics of diabetes and of IR. Among monzygotic twins, the concordance of type 1 diabetes is 33–50% and that of type 2 diabetes 90%. The risk of type 2 diabetes is 7–14% if one parent is affected but 50% with two parents. Siblings are threefold more likely to develop type 2 diabetes. The Maturity Onset Diabetes of the Young (MODY) autosomal
dominant disorders account for 1–5% of diabetes and are caused by a variety of genes affecting insulin secretion. Rotter noted that $h^2$, a measure of the proportion of phenotypic variation in a population attributable to genetic variation, has been reported to range from 17 to 53% for fasting insulin and from 25 to 76% for insulin sensitivity, with $h^2$ of 58% for insulin clearance. These genetic variations in insulin levels and action are in turn associated with obesity, polycystic ovary syndrome, nonalcoholic steatohepatitis, CVD, and hypertension. A number of candidate genes have been studied. There are rare mutations in peroxisome proliferator-activated receptor (PPAR)γ causing monogenic diabetes, but the common Pro12Ala polymorphism is also associated with type 2 diabetes, with the more common Pro allele increasing diabetes risk. A group of linked lipoprotein lipase alleles termed haplotype 1 is associated with insulin sensitivity, while haplotype 4 is associated with IR (2). Another linkage is with the Calpain10 (CAPN10) gene (3,4), with the haplotype 112 polymorphism associated with IR and also with decreased insulin secretion.

A genome-wide association study (GWAS) involves rapidly scanning markers across the complete genomes of many people to find genetic variations associated with a particular disease. A number of such studies have been carried out over the past 5 years. Although there are 10,000,000 single nucleotide polymorphisms (SNPs) in the genome, 500,000 SNPs are sufficient to recognize 80% of variants, using haplotype/linkage disequilibrium analyses to capture variations in inherited groups of genes because not all genes are inherited independently (5). In 2007, six different GWASs for type 2 diabetes analyzed, in aggregate, 19,000 individuals (6,7), identifying 12 genes, most of which were related to the β-cell. Two of the genes were related to IR, PPARG, and the fat mass and obesity associated gene (FTO). The effect of FTO is expressed through increased BMI, and its gene products may influence appetite (8).

With the availability of multiple GWASs, meta-analysis has been used to combine information across datasets (9), imputing approximately 2 million SNPs that were not genotyped by making use of sequencing data from the Human Genome Project. With this approach, six additional loci were identified, none of which were related to IR, leading to the supposition that IR is necessary but not sufficient and that the majority of individuals with IR will not have diabetes so that only with loss of β-cell compensation will diabetes occur (10). This concept does not, however, imply that IR is not under genetic control. The IR locus IRS1 (11) and loci in the TCF7L2, CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), and Wolfram syndrome 1 (WFS1) genes have all been found with such studies. Another approach has been to carry out GWAS using fasting glucose, rather than the presence of type 2 diabetes, as the marker. With this approach, the glucokinase regulator (GCKR) gene was found to have polymorphisms associated with IR, although oddly with lower triglyceride levels; another gene is the melatonin receptor 1B gene (MTNR1B) (12). A meta-analysis of fasting glucose, homeostasis model assessment (HOMA) of β-cell function, fasting insulin, and HOMA of IR in 46,186 nondiabetic individuals, replicated in 76,558 individuals from 21 GWAS, identified nine loci for fasting glucose and one related to fasting insulin and HOMA of IR near the IGF1 gene (13). Further study led to identification of 12 genes, identifying 1 related to IR, Kruppel-like factor (KLF) 14, a transcription factor acting via maternal but not paternal inheritance. Rotter observed that fasting insulin reflects physiologic processes other than IR and so is a relatively crude measure, with heritability less than that for insulin sensitivity based on the minimal model analysis of the intravenous glucose tolerance test (14). The dilemma is the difficulty of direct measurement of insulin sensitivity, but Genetics Underlying Diabetes in Hispanics (GUARDIAN) is being undertaken to perform a GWAS to detect loci underlying variation in glucose homeostasis traits in a multicohort pedigree-based set of 4,685 Hispanics, with reports planned to be available during the coming year.

Philippe Froguel (London, U.K.) discussed genetic variations contributing to IR, discussing the concept that currently “all diabetics are treated the same” but that “personalized medicine approaches” are required in diabetes, which will require stratifying patients, eventually using genetic analysis (15). GWASs have, Froguel noted, discovered a great many genes associated with modest increase in diabetes risk. Some of these genetic variants are associated with IR, most via effects on obesity, but most appear to act on insulin secretion. Furthermore, he pointed out, the GWAS results pointing to the IRS1 locus appear to be derived from variants located ~500 kb upstream, although associated with decreased basal muscle insulin receptor substrate (IRS)-1 protein, and with impaired insulin-stimulated muscle phosphatidylinositol 3-kinase (PI3K) activity, without effect on insulin secretion (11). The locus near the melatonin receptor (12) is, he said, of interest given the association between changes in diurnal cycles and IR. Discussing GWAS for fasting glucose, he noted that GCKR is associated with C-reactive protein levels and therefore may be related to IR as well. Genetic susceptibility to type 2 diabetes may be modified by obesity status so that BMI-based case selection may help to find more type 2 diabetes loci; interestingly, the TCF7L2 effect size is greater in lean than in obese individuals. Another approach being carried out is analysis of cosegregation of alleles associated with diabetes among family members; such analyses have been carried out in MODY.

C. Ronald Kahn (Boston, MA) received the Distinguished Leader in Insulin Resistance Award of the WCIRCD and discussed aspects of the development of IR. There are, he said, more than 10 IRS proteins in the insulin signaling pathway, which activate PI3K, producing phosphatidylinositol (3–5)-trisphosphate, as well as activating the Akt family. Considering these three nodes, each one with alternate isoforms, regulatory peptides, and other controls, gives >1,800 potential combinations, some leading to glucose uptake and others to lipid synthesis or having alternative metabolic effects, expanding on the possibility brought up by Reaven (see Part 1 of this report [16]) that insulin sensitivity or resistance occurs to different extents in different tissues. Other pathways exist as well: the Ras/mitogen-activated protein kinase pathway, the CAP/cbl pathway regulating muscle and adipose tissue glucose uptake, and some twenty other less well-understood proteins, some of which are involved in the function of the cellular calveolar system. Other proteins deactivate the cascades initiated by insulin, such as protein tyrosine phosphatase 1-B, sirtuins, and mitogen-activated protein kinase phosphatase 4, “so when you put all of this together we have an enormously complicated challenge.” Further, Kahn commented, “we need to put [this]… in the context of cell-to-cell communication.” Adipocytes are associated with inflammatory macrophages, with both secreting cytokines activating receptors at distant sites, particularly skeletal muscle and liver. Adipocyte free
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Kahn described his studies to selectively reduce or eliminate the activity of specific genes in specific tissues as follows: “to look at what is IR one tissue at a time, or one pathway at a time.” Liver insulin receptor knockout (LIRKO) mice have no insulin signaling in liver and have a metabolic phenotype of hepatic IR, with the liver resembling that of type 1 diabetes, having increased activity of gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase, while gluco- kinase and l-pyruvate kinase activities are decreased. The IRS proteins are differentia- tional mediators of insulin action in liver, with IRS-1 activating PI3K and in turn leading to activation of Akt and stimulating PEPCK, resulting in increased gluco- neogenesis. Lipid synthesis depends both on IRS1 and IRS2, requiring not only Akt but also the atypical protein kinase C proteins λ and ζ, whose differential levels of activity may promote different phenotypes. Considering the relative roles of IR versus hyperinsulinemia in dyslipi- demia, Kahn discussed analyses of the LIRKO mouse, which fails to express he- patic insulin receptors, making the insulin level irrelevant (18). This model has lower cholesterol and triglyceride than controls but exhibits gene-environment interactions, with marked increase in cholesterol levels and elevations in VLDL and small dense LDL cholesterol on a high-fat diet and with low HDL in association with ac- celeration of atherosclerosis. The fact that this model of hepatic IR leads to hepatic steatosis and gallstone formation is, Kahn said, “a key component,” although he noted that “it’s not enough to lead to the hypertriglyceridemia.”

It is interesting that the relationship between obesity and IR is imprecise; at a BMI of 30 kg/m², some individuals have high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while others have low insulin sensitiv- high while o
more effective in weight loss, with greatest lipid benefits on the Mediterranean diet and, among diabetic patients, greatest glycemic and insulin-lowering effects on the low CHO diet (25). Gardner’s group used diets varying from very low to very high CHO in 311 overweight premenopausal women (26). Again, there was not “a lot of weight loss in any group,” but the lowest CHO group did best. When 21% fat and 63% CHO diets were compared with 55% fat and 17% CHO diets, participants were able to maintain the low CHO intake, leading to greater weight loss. There was, however, extreme heterogeneity in response at 12 months in his study. Gardner suggested this to be particularly important if one considers the heterogeneity of insulin sensitivity among obese individuals, considering whether one should recommend different diets for different patient characteristics. In Gardner’s study, characterizing patients by fasting insulin tertile, the most insulin-sensitive group did equally well on low-CHO and low-fat diets, while in the more insulin-resistant group the low-fat high-CHO diet lessened weight loss.

A number of additional observations suggest that characterizing patients by insulin sensitivity may predict response to diets of different composition. In a study of 73 obese young adults with intensive dietary intervention for 6 months and subsequent 12-month follow-up, those with higher insulin concentration at 30 min after oral glucose had greater 18-month weight loss and reduction in body fat with a low–glycemic load diet than with a low-fat diet, while weight loss was similar with the two diets in those with lower insulin response, suggesting that insulin-resistant individuals may particularly benefit from a low-CHO diet (27). HDL cholesterol and triglyceride levels improved more on the low-CHO diet, while LDL cholesterol decreased more with the low-fat diet. This difference between low-fat and low-CHO diets was confirmed in a metabolic ward study, with insulin-sensitive individuals losing more weight on the former and insulin-resistant individuals losing more on the latter diet so that the concept of “focus on calories” may require reassessment with better patient characterization (28).

Greater weight loss among overweight individuals with high 30-min insulin levels on low–glycemic load diets and a trend to greater weight loss with low 30-min insulin levels on a high–glycemic load diet were also reported in a study finding no predictive effect of fasting insulin using the IR index of HOMA measure (29). In a study of 181 participants in Gardner’s study, greater dietary adherence was (as expected) associated with greater weight loss (30), but interestingly insulin-resistant and -sensitive individuals had similar adherence on the low CHO diet, whereas the insulin-resistant patients had lower adherence to a low-fat high-CHO diet. Gardner pointed out that the low-fat high CHO diet resembles that given in U.S. national dietary recommendations, noting that insulin-resistant individuals may find it inherently more difficult to adhere to such a diet or, alternatively, that realizing that they do poorly on such a diet, they fail to follow it.

In the Quebec Family Study, individuals with higher 30-min insulin levels had greater 6-year weight gain when following a low-fat high-CHO diet but with higher dietary fat (31). Gardner reviewed further studies showing differences in gene profile comparing those doing better with low-fat versus low-CHO diet. In these analyses, all four diets were similar in overall weight loss but there appeared to be a low-CHO diet-best genotype and a low-fat best genotype, with a sixfold difference in weight loss (1–2 vs. 6 kg), retrospectively comparing individuals assigned to the genetically incorrect versus correct group. “This story will only expand,” he noted, as more genetic markers are identified, allowing us to identify relevant dietary macronutrient compositions for specific individuals so that it will not be “only calories.” Is there a threshold or is there a continuous response to gradients in dietary CHO? Is low CHO (or low fat) <40, <30, or <20%? What of different types of CHO, different fiber content, saturated versus poly- versus monounsaturated fats, and fish oil? All of these factors will need to be better understood in accurately devising appropriate dietary strategies for different individuals, but crucial, Gardner suggested, will be the realization that “diets work differentially” related to IR and to genetic predisposition. An interesting suggestion by Reaven was that insulin-resistant individuals on high-CHO diets may develop hyperinsulinemia-induced sodium retention.

Walter Pories (NC) discussed bariatric surgery and the mechanisms underlying resolution of diabetes after such procedures. He reviewed 16-year data with 95% follow-up of 608 patients who lost from 211 to 317 pounds. Approximately 20% had type 2 diabetes, and another 20% had IGT; after a mean follow-up of 9.4 years, 83 and 99%, respectively, were euglycemic. Such findings have, Pories stated, been confirmed by a number of studies (32), and medication requirements for other obesity-related conditions also decrease after surgery, with improvement in urinary incontinence, sleep apnea, and many other abnormalities occurring at different time rates. Pories cited further studies suggesting that operated individuals have reduction in malignancies, infections, musculoskeletal symptoms, and CVD (33).

Gastric bypass creates a very small gastric reservoir, delaying peptic emptying, with undigested food in the distal small bowel increasing glucagon-like peptide (GLP)-1 and peptide YY levels. Other factors potentially related to weight loss include reduction in the admixture of food with biliary and pancreatic secretions and changes in gastric secretion and in levels of gastric-derived peptides. Of procedures currently in use, bypass is of greater efficacy than gastric restriction (34).

Addressing the question of danger of surgery, Pories acknowledged that procedures carried out in the past were not safe and that “you do have to take care of these patients,” noting that some hospitals fail to have acceptable quality control. The Surgical Review Corporation was created to certify Bariatric Centers of Excellence, and major insurers currently will not reimburse procedures performed by hospitals not in the program. Mortality rates at 30 and 90 days are 0.09 and 0.11%, which are below the 3.5% level following coronary artery bypass grafting and the 0.3% level following hip replacement and comparable with the 1% level after labor and delivery (35). Pories commented that surgical mortality should be seen as an important indicator of quality (36) and reviewed a comparison of 154 individuals undergoing gastric bypass with 78 refusing operation, showing overall and 1-year mortality of 9 vs. 28% and of 1 vs. 4.5% per year, respectively. Of course, he acknowledged that “none of these are prospective randomized trials.”

Weight regain is usually, he suggested, a technical problem caused by gastro-gastric fistula, stretched pouch, or a short intestinal limb, although occasionally patients have emotional problems causing weight regain. Early complications are those of all surgical procedures, including bleeding and infection, but specific late complications include nutritional
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deficiencies, emotional problems, and internal hernias. Pories concluded that bariatric surgery should be recommended for individuals with BMI >35 kg/m² with comorbidities or >40 kg/m² without comorbidities, pointing out that currently only 1% of eligible patients have the surgery.

Of course, one must realize the caveats to Pories’ position. Allocation bias is likely in analyses comparing individuals choosing to have surgery with those who chose not to have surgery. There are likely to be unmeasured differences including health care insurance, social support, education, and overall willingness to adhere to medical recommendations, all of which can be of major importance in outcome, particularly when long-term follow-up is incomplete with overrepresentation of successful patients. In a recent study giving median 13-year outcomes of laparoscopic banding, only 82 of 151 operated patients had adequate follow-up, of whom 22% had minor and 39% major complications, the latter including 28% with band erosion, with a 60% reoperation rate and neutral benefit in quality-of-life score (37). In another study, suicide rates following bariatric surgery were found to far exceed age- and sex-matched rates, with the majority of events occurring after 6 months (38); although not necessarily related to the surgery, it is clear that we need more information before recommending that these procedures be carried out more often, as Pories seemed to recommend.

Sun Kim (Stanford, CA) discussed β-cell function after gastric bypass, reviewing the rare condition of hyperinsulinemic postprandial (but not fasting) hypoglycemia (39). This typically occurs several years after gastric bypass and results in significant hypoglycemia with neuroglycopenia, having been reported in ~50 patients. A recent Swedish registry found hypoglycemia requiring hospitalization in 9 of 5,040 patients who had gastric bypass and in no patients after restrictive procedures (40).

Initial suggestions that the condition is caused by nesidioblastosis, based on a report of six patients with islet cell enlargement and dysplasia with β-cell budding from ductal epithelium (41), have been reinterpreted by others to suggest that the β-cell enlargement found in these patients correlates with the degree of obesity (42), implying that after gastric bypass the β-cell does not compensate for the improvement in insulin sensitivity. Kim reviewed her study comparing patients after gastric bypass with and without hypoglycemia and with nonbypass control subjects in the lowest, intermediate, and highest insulin-sensitivity tertiles (43). After a glucose load, both bypass groups had hyperinsulinemia, with higher peak glucose and lower glucose nadir and with one-third of bypassed patients showing post–glucose load hypoglycemia, the latter proportional to the degree of the glucose peak; this relationship was not seen in obese nonoperated control subjects. Symptomatic hypoglycemic patients appeared to show a pattern similar to that of insulin-sensitive control subjects (44), suggesting that there is no intrinsic β-cell abnormality. Interestingly, she found elevated levels of GLP-1, perhaps explaining some aspects of the patients’ hypoglycemia; other studies after gastric bypass also show progressively earlier peak glucose and peak insulin over time, with higher peak levels of GLP-1 (45). Why some individuals develop profound symptomatic hypoglycemia after gastric bypass is, however, unclear.

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